Song evolution and diversification patterns are uncorrelated in honeyeaters

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ABSTRACT

Birdsongs are well-known acoustic signals and are thought to play a key role in sexual selection. Despite the established role of birdsong in sexual selection and reproductive isolation at microevolutionary scales, its macroevolutionary impacts on clade diversification remain largely unexplored. Here, we test the hypothesis that shifts in song evolution influence diversification using honeyeaters, a diverse clade of songbirds restricted to Australasia. Using song data for 163 honeyeater species, we employ phylogenetic comparative models of trait evolution to detect shifts in optima for song evolution and use hidden state-dependent speciation and extinction models to assess the influence of these shifts on diversification. Contrary to our expectations, we find no correlation between shifts in song evolution and shifts in diversification rates, and models suggest other factors play a role. Overall, shifts in song evolution do not significantly contribute to diversification in honeyeaters, likely due to other ecological factors influencing diversification in this group including range size and dispersal ability.

Keywords: sexual signals, speciation, extinction, macroevolution, RevBayes, trait evolution

INTRODUCTION

Determining what factors cause species diversification is a central goal of many evolutionary studies. The influence of morphological traits (Cooney & Thomas, 2021; Rabosky et al., 2013; Simões et al., 2020) and environmental factors such as ecological opportunity (Dumont et al., 2012; Mahler et al., 2010), on rates of speciation and diversification have been a major focus, but other factors including sexual signals, can also play a role (Maan & Seehausen, 2011; Ritchie, 2007; Wagner et al., 2012). Divergence and development of reproductive isolation are key initial phases in speciation events (Coyne & Orr, 2004; Hernández-Hernández et al., 2021; Jarvis, 2021). Sexual signals frequently emerge as the most divergent traits between closely related species and often experience strong selection (Ritchie, 2007; Servedio & Boughman, 2017). The evolution and divergence of sexual signals can outpace ecological divergence (Arnegard et al., 2010) and consequently it has been hypothesized that rapid changes in sexual signals could drive species diversification (Maan & Seehausen, 2011; Servedio & Boughman, 2017).

Vocalizations are in part sexual signals that have the potential to influence speciation. Acoustic signals are used by a wide range of organisms and are complex, behavioral traits that enable individuals of the same species to identify one another (Laidre & Johnstone, 2013). Acoustic signals have broad-scale functions in species recognition and fine-scale functions in mate choice, thus potentially influencing speciation (Catchpole & Slater, 2003; Wilkins et al., 2013). Divergence of acoustic signals has played a role in the early stages of speciation in a wide range of organisms including cicadas (Marshall et al., 2008), crickets (Gray & Cade, 2000; Tinghitella et al., 2018; Zuk et al., 2006), frogs (Boul et al., 2007), and birds (Grant & Grant, 2006). In the context of reproductive character displacement, however, the strength of selection on signals depends on range overlap, with sexual signals expected to be under greater divergence when species are sympatric rather than allopatric (Maan &

Seehausen, 2011). Therefore, at macroevolutionary scales (i.e., clade or family-level), the strength of selection on acoustic signals is expected to vary between species and among clades. It remains unclear whether the processes of divergence, assortative mating, and selection of acoustic traits, which occur at the microevolutionary scale, can have macroevolutionary consequences for clade diversification.

Birdsong is one of the best-known acoustic signals and is an important sexual signal. Male birds sing to attract females: consequently, song can act as an important premating isolation barrier and influence speciation (Price, 2008; Tobias et al., 2020). Indeed, it has been long proposed that song evolution could explain the diversity of passerine birds (Baptista & Trail, 1992; Fitzpatrick, 1988). The role of birdsong in speciation and reproductive isolation has been studied at fine scales, and empirical evidence supports theoretical expectations. Divergence of song has contributed to reproductive isolation in warblers (Irwin et al., 2001), and is thought to have influenced the early stages of peripatric speciation in antbirds in Amazonia (Seddon & Tobias, 2007). Additionally, long-term studies on Darwin's finches have demonstrated that sexual selection on differences in vocalizations, resulting from changes in beak shape morphology, have impacted mate choice and contributed to reproductive isolation and speciation (Grant & Grant, 2006; Podos, 2001; Podos & Nowicki, 2004). Although acoustic signals are commonly hypothesized to influence diversification (Wilkins et al., 2013), most studies have focused on population or sister species comparisons, and empirical studies testing the influence of signal divergence on speciation at broader scales remain scarce (Mason et al., 2017). Where the relationship between song and speciation has been investigated at macroevolutionary scales, bursts in rates of speciation coincide with bursts in song evolution, however, no significant correlations have been uncovered at the family-level (Mason et al., 2017), with the important caveat that bursts in song evolution were examined via shifts in evolutionary rates measured

with Brownian motion models, rather than via more complex models that can incorporate changes in optima position (Uyeda and Harmon 2014).

The relationship between diversification and birdsong has proven increasingly difficult to disentangle, largely due to song itself being a complex trait, which in birds is influenced by developmental mode and constrained by factors such as beak morphology, body size, and the environment (Derryberry et al., 2018; Friedman et al., 2019; Friis et al., 2022). The majority of macroevolutionary studies on birdsong have therefore focused on the underlying drivers of song evolution. Like most sexual signals, acoustic signals are expected to diverge between close relatives, especially if ranges overlap, potentially leading to a lack of phylogenetic signal in the trait (Pfennig & Pfennig, 2010). However, song frequency is understood to be phylogenetically conserved (Mikula et al., 2021), largely due to constraints imposed by morphological traits, especially body size (Derryberry et al., 2018; Pearse et al., 2018). Moreover, the developmental mode of song is thought to influence song evolution and could complicate its relationship with speciation. Birdsong ranges from learned to innate, and learning is understood to be a form of phenotypic plasticity which is influenced by cultural transmission and can impact evolutionary outcomes (Pfennig et al., 2010). Learned songs are typically associated with higher rates of evolution (Mason et al., 2017) and divergence (Lachlan & Servedio, 2004) than innate songs, although conflicting evidence exists for this (Freeman et al., 2017). Such complexity in the evolution of acoustic traits provides a challenge for diversification approaches and can result in disparity when investigations are conducted over varying phylogenetic and spatial scales.

To overcome such limitations, we test the relationship between song and diversification using a monophyletic clade restricted to a particular region, recognizing the power that focusing on a diverse single clade can bring (Marquet et al., 2004). Thus, we focus

on honeyeaters (Aves: Meliphagidae), a phenotypically diverse clade of birds (192 species; Gill et al., 2020) that range throughout Australasia and are known for their distinctive vocalizations (Higgins et al., 2008). Song in honeyeaters is learned and varies greatly among species, with some species exhibiting simple and monotonal songs, whereas others have complex and variable songs (Fig. 1). This variation in vocalizations, combined with the high diversity and endemism of honeyeaters, makes this group an ideal model for studying the evolution of vocalizations and their potential role in species diversification.

Specifically, we use honeyeaters as a model clade to test the hypothesis that shifts in song evolution are correlated with shifts in diversification. We predict that if song is a major driver of diversification, then shifts in song evolution toward evolutionary optima will promote speciation and be correlated with shifts in diversification. Using a dataset of five song variables for 163 species of honeyeater, we model song evolution and identify shifts in song evolution, and then test whether these shifts are associated with significant increases in rates of diversification.

METHODS

Song and phylogenetic data

We use a dataset of male honeyeater song traits from a previous study (Hay et al., 2024). Containing data for 163 honeyeater species, the song dataset considers five relevant aspects of song (Fig. 1; Table S1) which have commonly been explored in past song studies (Derryberry et al., 2018; Mason & Burns, 2015; Mason et al., 2017): peak frequency (frequency which the most sound energy was produced), maximum frequency (maximum frequency of the highest pitched note in song), minimum frequency (minimum frequency of the lowest pitched note in song), song bandwidth (difference between maximum and

minimum frequency), and song pace (number of notes/song duration). Song variables were $\log_{10}$-transformed prior to statistical analysis to meet parametric assumptions of normality and homogeneity of variance, and because logarithmic scales of sound frequency correspond to how birds perceive and modulate sound (Cardoso, 2013).

To model trait evolution and estimate speciation rate we used a honeyeater phylogeny from Hay et al. (2022), which contains 192 species of honeyeater. The phylogeny was trimmed to represent the 163 honeyeater species for which we had song data and using the '*drop.tip'* function from the 'ape' package (Paradis et al., 2004) in R (R Core Team, 2013). Trait values are predicted to be more similar between closely related species, however sexual signals are often expected to be the most divergent traits between species (Servedio & Boughman, 2017). To test for how conserved song traits are, we used the '*phylosig'* function from the R package 'phytools' (Revell, 2012), and estimated the phylogenetic signal of each song variable using Pagel's lambda (Pagel, 1999) and a likelihood ratio test (Table S2). Pagels lambda is a scaling parameter that ranges from 0 to 1. Using this measure, a lambda of 0 indicates no correlation between species, and a lambda of 1 indicates that the correlation of trait values between species is equal to expectations of Brownian motion (Revell et al., 2008).

Analyses

A range of phylogenetic comparative methods, implemented through the program RevBayes (Höhna et al., 2016) were used to test the relationship between song and diversification. RevBayes enables Bayesian inference for phylogenetic comparative methods and uses a probabilistic graphical model framework which is a powerful generic framework for specifying and analyzing statistical models (Höhna et al., 2014; Höhna et al., 2016). First, to explore patterns of song evolution in honeyeaters, we used relaxed Ornstein-Uhlenbeck (OU) models of trait evolution to identify shifts in trait evolution of song traits in RevBayes.

Additionally, relaxed OU models were implemented in a multivariate framework using RevBayes and verified using multivariate OU models using the R package 'PhylogeneticEM' (Bastide et al., 2018). The influence of these shifts on diversification were then tested with hidden state-dependent speciation and extinction models (Beaulieu & O'Meara, 2016) in RevBayes (Höhna et al., 2016).

Relaxed OU models of trait evolution were used to model song evolution along the honeyeater phylogeny. Relaxed OU models allow the optimal phenotype to vary over branches of the phylogenetic tree to estimate tip-specific measures of OU evolution (Uyeda and Harmon 2014) and to infer branch-specific evolutionary optima of song evolution in honeyeaters. Here, we first assessed patterns of OU trait evolution in each of the five song traits (peak frequency, maximum frequency, minimum frequency, bandwidth, and pace) separately. Trait evolution is inherently a multivariate process (Revell & Harmon, 2022; Walsh & Blows, 2009), thus we also implemented multivariate relaxed OU models. The function '*dnPhyloOrnsteinUhlenbeckREML'* does not enable the inclusion of a correlation matrix to account for covariation between multivariate traits. To overcome this limitation, we performed a phylogenetic principal component analysis on the five song variables to ensure no correlation between traits, using the function *'phyl.pca'* from the R package "phytools" (Revell, 2012). We considered pPC1 to pPC3, which accounted for 97.6 % of the total variation, in analysis (Table S3). Relaxed OU models were implemented in RevBayes using reversible-jump Markov chain Monte Carlo and were executed through the CIPRES Science Gateway v3.3 online server (Miller et al., 2010). The MCMC sampler was set for 4 runs of 50,000 generations with a burn-in of 10%. Tracer v1.7.1 (Rambaut et al., 2018) was used to visualize log files and to ensure convergence. Because priors can influence performance of OU models, particularly the number of expected shifts, all models were repeated with three different priors on the number of shifts (15, 30, and 45). Model fit was assessed using

198 stepping-stone and path sampler analyses, and the best model was selected based on marginal
199 likelihood values. Branch-specific rates of relaxed OU evolution were visualized using the
200 '*plotTree'* function from the R package 'RevGadgets' (Tribble et al., 2021). Priors had little
201 effect on estimates, and all cases the preferred models had 45 shifts (Table S4; Fig. S1-S6).
202 Shifts in song evolution were determined based on tip theta values extracted from relaxed OU
203 models. Under an OU model, theta represents optimal values in which continuous characters
204 are assumed to evolve toward. Species were identified to have shifts in song evolution if their
205 tip theta value varied by more than one standard deviation from the mean. We also classified
206 species to have a shift if they fell within a clade in which all other species were associated
207 with shifts (Tables S5-S10).

208 As an additional measure to test for shifts in trait evolution, and to verify the results
209 from the multivariate relaxed OU models in RevBayes, we used the R package
210 'PhylogeneticEM' (Bastide et al., 2018). PhylogeneticEM simulates a scalar OU model of
211 evolution that allows for maximum likelihood estimation of shifts in OU trait evolution and
212 uses this approach to automatically detect position of shifts along a phylogeny, while
213 accounting for correlations among traits (Bastide et al., 2018). The function '*phyloEM'* was
214 used to test for OU shifts in multivariate trait evolution, using pPC1-pPC3, the same dataset
215 which was used for the multivariate OU model in RevBayes.

216 Finally, to test the influence of shifts in song evolution on diversification rates we
217 used hidden state-dependent speciation and extinction models (HiSSE; Beaulieu & O'Meara,
218 2016), implemented in RevBayes (Höhna et al., 2016). The HiSSE framework enables testing
219 of character dependent diversification and additionally models an unobserved hidden
220 character that potentially impacts species diversification rates. HiSSE models test the
221 influence of binary characters on speciation and extinction rates and can disentangle rate

variation due to the character of interest with the inclusion of a hidden state. HiSSE represents a substantial improvement on previous state-dependent diversification models (Maddison & FitzJohn, 2015; Rabosky & Goldberg, 2015). Because HiSSE is restricted to testing the influence of binary characters on clade diversification, species associated with shifts in song evolution were coded with a '1' and all other species were coded as '0' (Tables S5-S10). This was done to test whether shifts in song evolution are associated with differential diversification rates. Speciation and extinction models can be subject to bias when species are missing (Mynard et al., 2023), therefore we implemented HiSSE models across the full honeyeater phylogeny containing 192 species of honeyeater (Hay et al., 2022). The 29 honeyeater species with no song data were treated as having unknown tip states and were coded with '?' rather than '0' or '1'. The MCMC sampler was set for 4 runs of 3,000 generations and a burn-in of 10%. Tracer v1.7.1 (Rambaut et al., 2018) was used to visualize output and determine convergence. The R package 'RevGadgets' (Tribble et al., 2021) was used to visualize model output with the '*plotTrace*' function, and summary statistics were calculated using *'summarizeTrace'* (Table S11).

RESULTS

Phylogenetic signal varied substantially among the five song variables. Values of Pagel's lambda ranged from 0.31 to 0.92 and the significance values of the likelihood ratio tests were all below $p < 0.001$ (Table S2). Song bandwidth had the lowest signal ($\lambda = 0.31$), followed by maximum frequency ($\lambda = 0.58$), pace ($\lambda = 0.66$), peak frequency ($\lambda = 0.75$) and then minimum frequency ($\lambda = 0.92$).

Univariate relaxed OU models revealed several patterns in song evolution. Peak frequency and maximum frequency had very similar estimates for relaxed OU models of trait evolution. Here, the clades *Myzomela* + *Sugomel* (including *Lichmera lombokia*) and

Philemon were associated with shifts in song evolution for peak and maximum frequency (Fig. 2a and b; Tables S5-S6). Minimum frequency also contained similar estimates to peak and maximum frequency but did not uncover a shift for *Philemon* (Fig. 2c; Table S7). Despite this, we found little evidence that shifts in trait evolution influenced honeyeater diversification rates for peak frequency (mean $\text{diversification}_{\text{no shift}}$ = 2.38, 95 % CI = [1.80, 3.15]; mean $\text{diversification}_{\text{shift}}$ = 3.42, 95 % CI = [2.29, 4.88]; Fig. 2a; Table S11), maximum frequency (mean $\text{diversification}_{\text{no shift}}$ = 2.41, 95 % CI = [1.82, 3.20]; mean $\text{diversification}_{\text{shift}}$ = 3.39, 95 % CI = [2.28, 4.87]; Fig. 2b; Table S11), and minimum frequency (mean $\text{diversification}_{\text{no shift}}$ = 2.47, 95 % CI = [1.87, 3.23]; mean $\text{diversification}_{\text{shift}}$ = 3.93, 95 % CI = [2.34, 6.11]; Fig. 2c; Table S11). There was also no evidence that shifts in song bandwidth evolution influenced rates of diversification (mean $\text{diversification}_{\text{no shift}}$ = 2.67, 95 % CI = [2.04, 3.49]; mean $\text{diversification}_{\text{shift}}$ = 2.64, 95 % CI = [0.72, 4.82]; Fig. 2d; Table S11). Interestingly, we found shifts in pace evolution to be associated with lower rates of diversification (mean $\text{diversification}_{\text{no shift}}$ = 2.78, 95 % CI = [2.11, 3.75]; mean $\text{diversification}_{\text{shift}}$ = -0.72, 95 % CI = [-6.91, 0.36; Fig. 2e; Table S11) and this was statistically significant, however, this result could be due to the lower sample size for species associated shifts in pace evolution (7 species).

The two methods of multivariate OU trait evolution (RevBayes and PhylogeneticEM) uncovered relatively similar shifts in song evolution for honeyeaters. Species from the genera *Philemon, Myzomela*, and *Meliarchus* were associated with shifts in OU trait evolution for both approaches (Fig. 3a; Table S10; Fig. S7). The PhylogeneticEM method did reveal a shift associated for the larger clade containing *Ptilotula*, *Anthochaera*, *Melidectes*, and relatives, which was not recovered in the RevBayes relaxed multivariate OU model (Fig. 3a; Table S10; Fig. S7). Both methods had similar estimates for diversification and speciation rates (Fig. 3b and c; Table S11). Nevertheless, we found no evidence that shifts in song evolution

were associated with increased rates of diversification for both the RevBayes (mean diversification$_{no\ shift}$ = 2.40, 95 % CI = [1.80, 3.24]; mean diversification$_{shift}$ = 3.49, 95 % CI = [2.37, 5.04) and PhylogeneticEM approaches (mean diversification$_{no\ shift}$ = 2.18, 95 % CI = [1.56, 3.05]; mean diversification$_{shift}$ = 3.31, 95 % CI = [2.38, 4.77]), and the 95 % confidence intervals overlap substantially (Fig. 3b and c; Table S11).

DISCUSSION

In this study, we used honeyeaters to explore the macroevolutionary consequences of song evolution, a sexually selected behavioral trait, which has commonly been hypothesized to influence speciation in birds. We found no evidence that shifts in song evolution were associated with increases in diversification rates. The significant overlap between confidence intervals of states is present in all tests, except for song pace, and hidden state-dependent speciation and extinction models suggest that there are other factors (hidden states) which play a role. Ultimately, shifts in song evolution do not appear to contribute to diversification in honeyeaters, possibly due to other factors including geographical processes and dispersal to islands dominating speciation history in honeyeaters (Hay et al., 2022), and any apparent influence of song is likely mediated by these factors and underlying variation in body size.

Most of the interspecific diversity of song frequency and pace in honeyeaters can be explained by evolutionary history. Theory suggests that divergence is expected for sexually selected traits between closely related species (Maan & Seehausen, 2011; Servedio & Boughman, 2017). Thus, the level of phylogenetic signal uncovered for frequency and pace is contrary to such theoretical expectations. This is unsurprising, however, given other studies have found similar levels of phylogenetic signal for song traits in birds: peak frequency was found to have a lambda of 0.87 for 5085 species of passerine birds (Mikula et al., 2021), and

a lambda of 0.74 for 1022 passerine species (Friis et al., 2022). Our results are consistent with these previous studies and confirm that peak frequency of song is phylogenetically conserved. This is likely driven by allometric constraints imposed by body size (Fletcher, 2004), which itself is a conserved trait and directly impacts the length of the vocal tract and the size of the syrinx (Bertelli & Tubaro, 2002; Suthers & Zollinger, 2008). Certainly, body size has been demonstrated to have a prominent influence on honeyeater song and is understood to structure variation in peak frequency and pace (Hay et al., 2024). Interestingly, minimum frequency was found to have the highest phylogenetic signal rather than peak frequency. It is unclear if this is the case in other clades as past studies have typically focused on peak frequency. Nonetheless, body size is understood to place a limit on the lowest frequency that a bird can effectively produce (Fletcher, 2004; Friis et al., 2021), so perhaps minimum frequency is particularly constrained by body size.

We found no evidence that shifts in song evolution were associated with higher rates of diversification in honeyeaters. Macroevolutionary influences of song evolution on speciation has been explored in 581 species of tanagers and ovenbirds and shifts in song were found to be correlated with shifts in speciation, but this was not significant when each family was considered separately (Mason et al., 2017). This is consistent with the findings of this study, which is restricted to the family-level, and could imply that macroevolutionary signals of song on diversification emerge only when very different clades of birds are examined simultaneously, as is the case for other taxa too (Rabosky & Huang, 2016; Rabosky et al., 2013). Additionally, the song dataset used in this study was missing data for 29 honeyeater species. As is often the case with missing data, species inhabiting the most remote areas were data deficient. This skewed our dataset towards common species and especially those found in Australia and New Zealand. Areas such as New Guinea, which has high honeyeater species richness (Marki et al., 2019), and islands throughout the Indo-Pacific, which contain endemic

species, were lacking in data. Given previous work highlighting island settings for speciation in the group (Hay et al., 2022), this potential effect of our dataset should be considered a caveat for the study.

We did find some evidence that shifts in song pace evolution influenced rates of diversification and shifts in song pace were correlated with lower rates of diversification. However, only seven species of honeyeater were associated with shifts in pace evolution, and this low sample size has likely influenced this outcome. Given that the number of species associated with shifts (7 species) is far lower than the number of missing species included in the model (29 species), we recommend caution when interpreting this result.

Nonetheless, our results indicate that shifts in song evolution are not major drivers of diversification in honeyeaters. Recent studies have found that faster rates of song evolution do not result in higher speciation rates in New World passerine birds (Freeman et al., 2022), and ecological and demographic factors may provide a better explanation for speciation (Freeman et al., 2022; Harvey et al., 2019; Singhal et al., 2018). State-dependent speciation and extinction models used in the present study indicated hidden states are associated with differential rates diversification. Thus, other factors not considered here are likely contributing to patterns of diversification. Honeyeaters have a dynamic evolutionary history: Australia is the center of honeyeater diversity (Driskell & Christidis, 2004; Norman et al., 2007; Oliveros et al., 2019), however, dispersal to islands throughout the Indo-Pacific, and vicariant speciation due to connection and disconnection of New Guinea and the changing biomes of Australia have played key roles in the diversification of this group (Andersen et al., 2014; Hay et al., 2022; Marki et al., 2017; Toon et al., 2010). Moreover, past studies have found range size and dispersal ability to significantly contribute to speciation in honeyeaters when applying state-dependent speciation and extinction models (Hay et al., 2022).

Allopatric speciation is the predominant mode of speciation in honeyeaters and could explain the lack of influence of shifts in song evolution on diversification because selection on signal divergence is expected to be stronger for sympatry (Pfennig & Pfennig, 2010). Songs are thought to diverge slowly in allopatry, and only rapidly increase on secondary contact, contributing to reinforcement (Lachlan & Servedio, 2004; Servedio & Boughman, 2017). Thus, contributions of song to speciation likely depends on the mode of speciation, so although song could be aiding microevolutionary processes such as reproductive isolation, other factors including range size and dispersal ability have contributed more to diversification (Hay et al., 2022).

Across all models of trait evolution, shifts in song evolution are notable in clades of *Myzomela* + *Sugomel* (including *Lichmera lombokia*) and *Philemon*. These clades have previously been identified to have increased rates of speciation (Hay et al., 2022) and are also the most speciose genera in honeyeaters (Gill et al., 2020). However, past studies have shown that small range sizes resulting from dispersal to islands is a primary driver of speciation in these clades (Hay et al., 2022), and the (non-significant) slight increase in diversification rates observed with these shifts in song evolution could be due to these underlying factors. It is also important to consider the influence of body size on these patterns because honeyeater song is under strong allometric constraints (Hay et al., 2024), and these clades show great differences in body size: all species of *Myzomela* weigh between 6 to 16 g, and species of *Philemon* range from 50 g to 194 g, in which the majority of species are over 100 g (Dunning, 2008; Tobias et al., 2022). Body size evolution has been correlated with speciation and species diversity across different vertebrate clades (Rabosky & Adams, 2012; Rabosky et al., 2013; Ricklefs, 2004), and has contributed to speciation in honeyeaters (Brennan & Keogh, 2018; Marki et al., 2019). Therefore, it is possible that the shifts in song evolution

could be due to shifts in body size evolution, and underlying variation in body size could be contributing to both the observed patterns of song evolution and diversification in this study.

Understanding the influence of reproductive isolation on diversification has been recognized as an urgent question in evolutionary biology (Matute & Cooper, 2021), especially because the evolution of reproductive isolation often defines speciation (Coyne & Orr, 2004). Here, we contribute to understanding of this question by testing the hypothesis that shifts in song evolution have influenced diversification in honeyeaters. We uncovered several shifts in song evolution but found no evidence that these shifts were associated with significant increases in diversification rates. Models also indicated that other factors (hidden states) contribute to differential rates of diversification, implying that diversification of honeyeaters is better explained by other factors.

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FIGURE CAPTIONS

Figure 1. Song spectrograms displaying frequency (kHz) over time (s) for five species of honeyeater: a) New Zealand Bellbird (*Anthornis melanura*; xeno-canto accession number = XC153005), b) Eastern Spinebill (*Acanthorhynchus tenuirostris;* xeno-canto accession number = XC490800), c) Blue-faced Honeyeater (*Entomyzon cyanotis*; xeno-canto accession number = XC439288), d) Noisy Friarbird (*Philemon corniculatus*; xeno-canto accession number = XC287056), and e) Singing Honeyeater (*Gavicalis virescens*; xeno-canto accession number = XC334251). The five song variables considered in this study are indicated with annotations in a): peak frequency represents the frequency of maximum amplitude across the entire song, maximum frequency is the highest pitched note in song, minimum frequency is the lowest pitched note in song, bandwidth is the difference between maximum and minimum frequency across the song, and pace is the number of notes over song duration. Birds are illustrations by Eleanor Hay.

Figure 2. Results from relaxed OU and HiSSE models for each of the five song traits; peak frequency (a), maximum frequency (b), minimum frequency (c), bandwidth (d) and pace (e), Phylogenetic trees display branch specific theta values from relaxed OU models in RevBayes. Dots at the tips of the trees indicate which species were identified as associated with shifts in song evolution and were tested to influence speciation rates. Respective diversification rates extracted from HiSSE models associated with the coding at the tips are displayed in the density plots to the right of each phylogeny.

Figure 3. Honeyeater phylogenetic tree (a), the branches of the phylogeny display estimated branch theta values from the multivariate relaxed OU model in RevBayes. Dots on the branches of the phylogeny indicate shifts revealed by PhylogeneticEM. Dots at the tip of the

tree show the binary classifications for HiSSE models from the RevBayes and PhylogeneticEM (labelled as PhyloEM) approaches. Density plots below the phylogeny show the estimated diversification rates extracted from HiSSE models for shifts identified by RevBayes (b) and PhylogeneticEM (c). Birds are illustrations by Eleanor Hay.

FIGURES

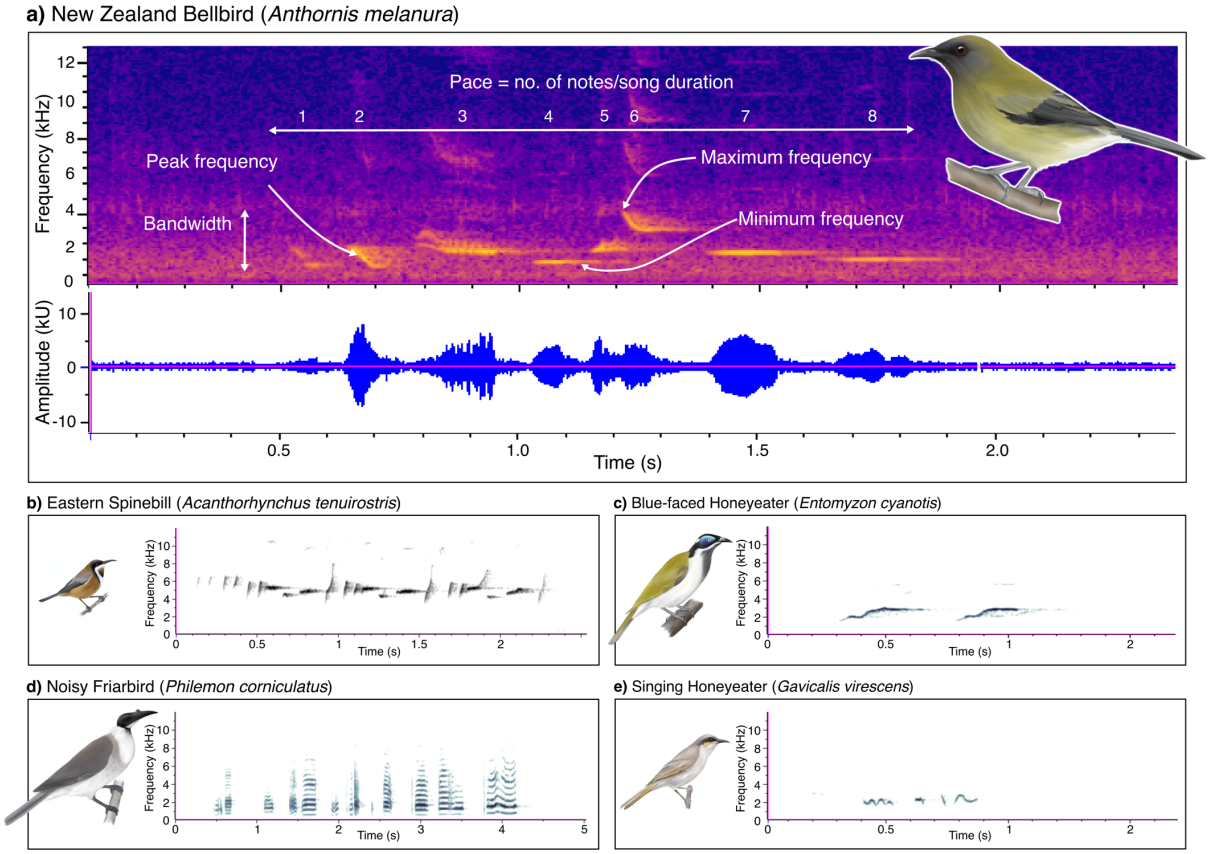

Figure 1.

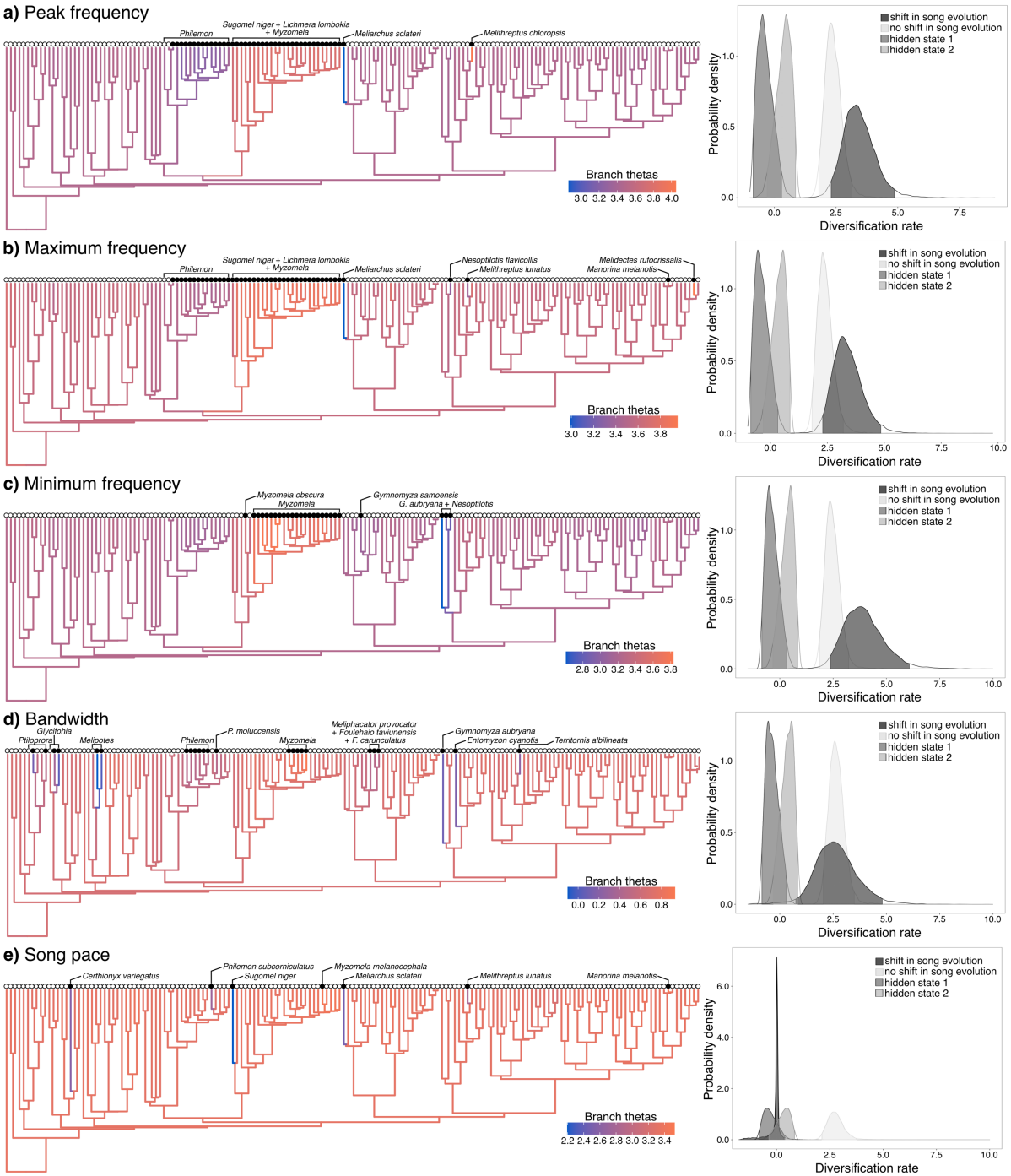
a) Peak frequency
Philemon
Sugomel niger + Lichmera lombokia + Myzomela
Meliarchus sclateri
Melithreptus chloropsis
Branch thetas
3.0 3.2 3.4 3.6 3.8 4.0
b) Maximum frequency
Philemon
Sugomel niger + Lichmera lombokia + Myzomela
Meliarchus sclateri
Nesoptilotis flavicollis
Melithreptus lunatus
Melidectes rufocrissalis
Manorina melanotis
Branch thetas
3.0 3.2 3.4 3.6 3.8
c) Minimum frequency
Myzomela obscura
Myzomela
Gymnomyza samoensis
G. aubryana + Nesoptilotis
Branch thetas
2.8 3.0 3.2 3.4 3.6 3.8
d) Bandwidth
Glycifohia
Philoprora
Melipotes
Philemon
P. moluccensis
Myzomela
Meliphacator provocator + Foulehaio taviunensis + F. carunculatus
Gymnomyza aubryana
Entomyzon cyanotis
Territornis albilineata
Branch thetas
0.0 0.2 0.4 0.6 0.8
e) Song pace
Certhionyx variegatus
Philemon subcorniculatus
Sugomel niger
Myzomela melanocephala
Meliarchus sclateri
Melithreptus lunatus
Manorina melanotis
Branch thetas
2.2 2.4 2.6 2.8 3.0 3.2 3.4
shift in song evolution
no shift in song evolution
hidden state 1
hidden state 2
Probability density
Diversification rate

Figure 2.

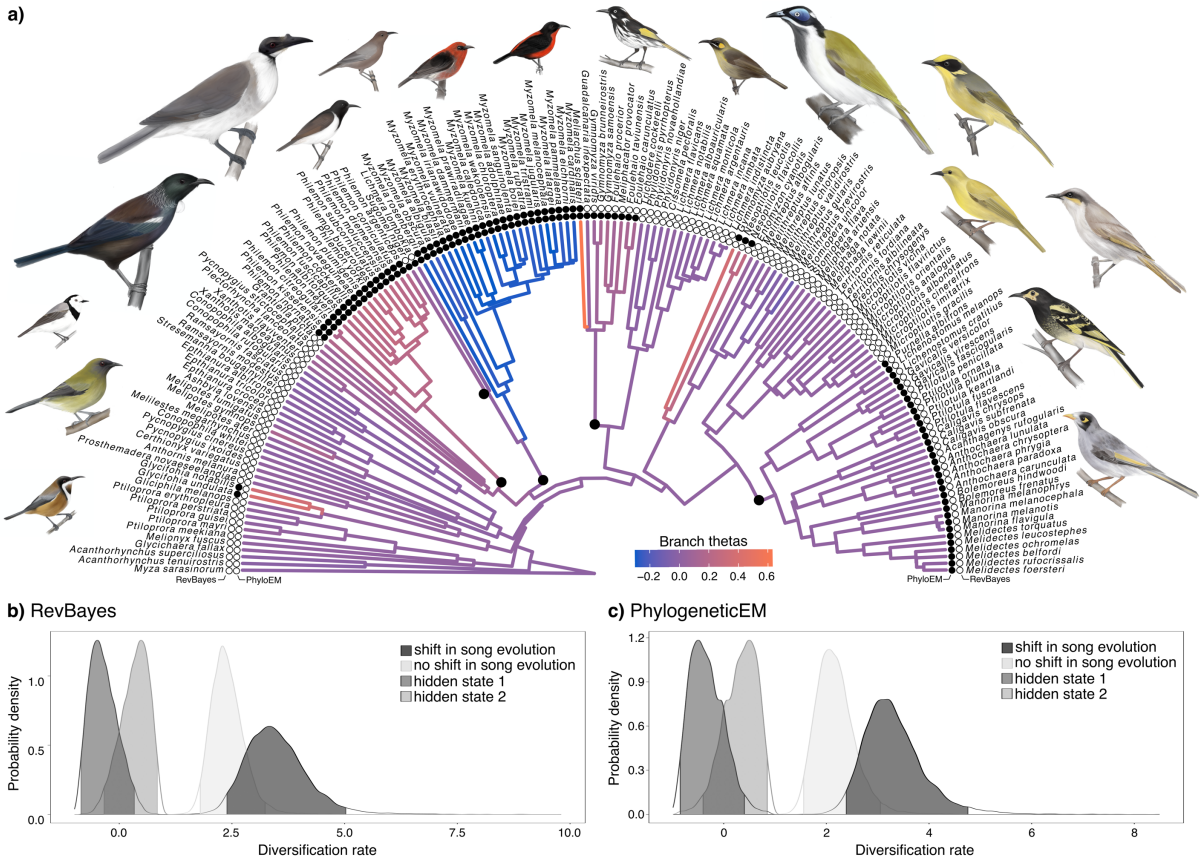
a)
Branch thetas
−0.2 0.0 0.2 0.4 0.6
RevBayes
PhyloEM
b) RevBayes
Probability density
Diversification rate
shift in song evolution
no shift in song evolution
hidden state 1
hidden state 2
c) PhylogeneticEM
Probability density
Diversification rate
shift in song evolution
no shift in song evolution
hidden state 1
hidden state 2

Figure 3.